Molecular and Biochemical Parasitology, 19 (1986) 51–59
Elsevier

MBP 00648

Studies on a filarial antigen with collagenase activity

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(Received 14 May 1985; accepted 13 November 1985)

We examined the ability of two filarial species, *Onchocerca volvulus* and *Brugia malayi*, to solubilize collagen molecules from native collagen fibrils. Collagenolytic activity was detected in extracts of adult worms, in living microfilariae of *O. volvulus* and in live infective larvae and adult female worms of *B. malayi*. Excretion–secretion factors produced in vitro by infective larvae of *B. malayi* also contained large amounts of collagenase. Studies with enzyme inhibitors suggest that the latter may be a metallo-protease. Antibodies to filarial collagenase were present in sera from patients with onchocerciasis and brugian filariasis and from mice immunized with *B. malayi*. These antibodies and a monoclonal antibody raised against *O. volvulus* antigens immunoprecipitate filarial collagenase but appear not to be directed against the active site of the enzyme.

Key words: *Onchocerca volvulus; Brugia malayi;* Collagenase; Protease; Antigen; Monoclonal antibody; Protease inhibitors

Introduction

The parasitic nematodes *Onchocerca volvulus* and *Brugia malayi* infect humans by invading the skin and migrating to host organs that are the final habitat of adult parasites: subcutaneous tissues for *O. volvulus*, lymphatic vessels for *B. malayi*. Most adult onchocerca reside within subcutaneous fibrotic nodules (onchocercomata), from which microfilariae escape to invade the dermis and other tissues, including the eyes [1,2]. Chronic microfilarodermia is associated with destruction of specialized dermal structures, particularly the interlacing strands of collagen and the elastica [3]. These pathologic changes result in loss of skin elasticity and lead to the so-called hanging groin syndrome [1].

The mechanisms by which filarial worms penetrate and migrate within host tissues remain largely unknown. Many years ago, Chandler postulated that parasites might release proteases for this purpose [4]. Since then, such enzymes have been described in various helminths and protozoa that invade host tissues, but the role of these nonspecific proteases remains unclear [5–9].

Because collagen and elastin are the major constituents of the extracellular matrix that are damaged in patients with chronic onchocerciasis, we examined whether collagenolytic activity is present in *O. volvulus* and in the related parasite species, subperiodic *B. malayi*. Our results show that filarial nematodes contain and release proteolytic enzymes that solubilize collagen molecules from native type I collagen fibrils. The collagenase is immunogenic in natural and experimental hosts of filariasis and shares antigenic epitopes with a bacterial collagenase.

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Abbreviations: EDTA, ethylenediaminotetraacetic acid: EGTA, ethyleneglycol bis-(β-ethyl ether)-tetraacetic acid; ELISA, enzyme-linked immunosorbent assay; E-S products, excretory–secretory products; HBSS, Hanks' balanced salt solution; L-Cys, L-cysteine; NEM, *N*-ethylmaleimide; OV5, monoclonal antibody to *Onchocerca volvulus* antigens number five; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, polyacrylamide gel electrophoresis with sodium dodecyl sulphate; STI, soybean trypsin inhibitor; TLCK, tosyl-lysine-chloromethylketone; TPCK, toluenesulfonyl-phenylalanine-chloromethylketone.

Materials and Methods

Parasites. O. volvulus. Onchocercomata and skin snips were collected in Sierra Parima, Venezuela, and cryopreserved as previously described [10]. Intact, but nonviable adult worms were prepared by digesting the nodules with collagenase [11]. Live microfilariae were recovered from skin snips as previously described [10]. All worms were washed extensively in phosphate-buffered saline pH 7.2 (PBS) before being used.

B. malayi. Adult worms and microfilariae of subperiodic *B. malayi* were obtained from peritoneal cavities of infected jirds as previously described [12]. Infective larvae of *B. malayi* were isolated from experimentally infected *Aedes aegypti* [13].

Ascaris lumbricoides. An adult worm obtained from an Indonesian patient was washed extensively in PBS and kept frozen at −20°C until used.

Parasite extracts. Parasite extracts were prepared as previously described [14], aliquoted and kept at −70°C until use. The protein content of these extracts was measured according to Bradford [15].

Excretory–secretory (E-S) products. To prepare E-S products, worms were incubated for 24–48 h in Hanks' Balanced Salt Solution (HBSS; Gibco Laboratories, Grand Island, NY). Pooled culture supernatants were filtered through a 0.45 μm membrane, concentrated in dialysis bags against Aquacide^R (Calbiochem, San Diego, CA), aliquoted and stored at −70°C until used. Metabolically labeled E-S products were similarly prepared by incubating worms in HBSS containing mixtures of radiolabeled amino acids (New England Nuclear, Boston, MA).

Control materials. Skin snips from Venezuelan donors without microfilarodermia were processed as described for infected skin snips. Homogenates of salivary glands from uninfected *A. aegypti* were prepared in the same manner as parasite extracts. Hemolymph from uninfected *A. aegypti* was collected by micropunture. Peritoneal fluid from infected and uninfected jirds was collected as described [12].

Collagenase assay. ^{3}H-labeled type I collagen was purchased from New England Nuclear. The fibril assay was performed as described by Terato et al. [16]. In brief, 0.5 μg labeled collagen was incubated for 2–3 h in Ca^{2+}-containing buffer to allow spontaneous fibril formation. Tissue extracts or worm products were then added and the mixture was incubated at room temperature for up to 36 h. All experiments were done in triplicate and included the following controls: buffer alone (to assess the stability of the preparation); trypsin (Sigma Chemical Co., 0.1 μg per assay; to assess the amount of denatured collagen present); *Clostridium histolyticum* collagenase (Sigma Chemical Co., 0.8 μg per assay); and *Rana catesbiana* collagenase (Sigma Chemical Co., 4 μg per assay). Enzyme activity was calculated by the formula:

$$\frac{\text{cpm released with enzyme} - \text{cpm spontaneous release}}{\text{cpm total} - \text{cpm spontaneous release}}$$

and is expressed as ng collagen solubilized in 24 h.

Human sera. Patient sera were collected from donors with onchocerciasis (Sierra Parima, Venezuela), mansonellosis (Orinoco Medio River, Venezuela) and brugian filariasis (Kalimantan Selatan, Indonesia) and stored at −20 °C until used. Sera from uninfected blood donors were obtained from the U.S. Red Cross.

Production of monoclonal antibodies. Monoclonal antibodies to *O. volvulus* were prepared by the fusion of SP-2 myeloma cells with spleen cells from hyperimmune BALB/c mice [17]. Antibody-producing hybrids were cloned by limiting dilution and expanded in vitro. Immunoglobulins in culture supernatants were concentrated by precipitation with 50% $(NH_4)_2SO_4$ [18].

Fractionation of sera and monoclonal antibodies. IgG-rich fractions were prepared by gel chromatography on CM-Affigel Blue columns (BioRad, Richmond, CA) from a pool of normal human sera, a pool of three sera from patients with onchocerciasis and concentrated hybridoma supernatants.

Antibodies to *C. histolyticum* collagenase were isolated from pooled onchocerciasis sera by affin-

ity chromatography with enzyme coupled to cyanogen bromide-activated Sepharose 4B according to the supplier's recommendations (Pharmacia Fine Chemicals, Piscataway, NJ). Purified enzyme was obtained from Sigma Chemical Co., St. Louis, MO (catalog no. C-0773 batch 114F-6805).

Detection of antibodies to C. histolyticum collagenase and to parasite antigens. Antibody titers were measured with a standard solid phase enzyme-linked immunosorbent assay (ELISA) using alkaline phosphatase-conjugated antisera to human and murine immunoglobulins.

Precipitation of C. histolyticum and filarial collagenase with antibodies. Antibody containing fractions eluted from CM-Affigel Blue and affinity columns described above were incubated overnight at 4°C with *C. histolyticum* collagenase or E-S factors of infective larvae of *B. malayi*. After precipitation of complexes by centrifugation (12000 × *g*, 15 min, 4°C), enzyme activity remaining in solution was determined as described above.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting of protein samples. Page (5–20%, w/v gels) was performed as described by O'Farrell [19]. Proteins were stained with silver nitrate [20]. Fluoroautoradiography was used to detect radiolabeled components [21].

Protein samples separated by SDS-PAGE were transferred electrophoretically to nitrocellulose paper [22]. Strips of these blots were incubated with human sera (diluted 1:80) or with concentrated monoclonal antibodies (diluted 1:5) as previously described [17].

Inhibitors. Phenylmethylsulfonyl fluoride (PMSF), *N*-ethylmaleimide (NEM), L-cysteine (L-Cys), ethyleneglycol bis-(β-ethyl ether)-tetraacetic acid (EGTA), ethylenediaminotetraacetic acid (EDTA), zinc chloride, copper sulphate and soybean trypsin inhibitor (STI) were obtained from Sigma Chemical Co., St. Louis, MO; tosyl-lysine-chloromethylketone (TLCK) and toluenesulfonyl-phenylalanine-chloromethylketone (TPCK) were purchased from Cyclo Chemical Corporation, San Diego, CA; fetal calf serum from Gibco Laboratories, Grand Island, NY. Purified platelet factor 4 was a gift from Dr. Paula Bockenstedt, Brigham and Women's Hospital, Boston, MA.

TABLE I

Extracts of adult nematodes contain collagenolytic activity

Species	Activity[a]	Percent inhibition by 10 mM EDTA
A. lumbricoides	1.44 ± 0.14(2)[b]	0.0
D. immitis	18.72 ± 0.01(2)	15.6
O. volvulus	194.40 ± 15.84(2)	28.0
B. malayi	21.60 ± 0.07(2)	15.9

[a] ng collagen solubilized at 25°C 24 h^{-1} (mg worm protein)$^{-1}$.
[b] Number of experiments.

Results

Filarial nematodes contain and release collagenolytic activity. In initial experiments, we examined whether aqueous extracts of whole worm homogenates contained collagenolytic activity. Extracts of adult *Dirofilaria immitis, O. volvulus* and *B. malayi* contained measurable enzyme activity, whereas an extract of *A. lumbricoides* did not (Table I). Solubilization of collagen molecules from preformed fibrils was partially inhibited by EDTA, a known inhibitor of some collagenases [24,25].

Next, we evaluated the ability of intact worms in different stages of development to solubilize native collagen. For this purpose, various num-

TABLE II

Collagenolytic activity of various developmental stages of filarial worms

Organism	Activity[a]	Percent inhibition by 10 mM EDTA
O. volvulus		
microfilariae	1.75 ± 1.11 (3)[b]	not done
B. malayi		
infective larvae	0.44 ± 0.12 (7)	45.9
adult male worms	0.00 ± 0.00 (2)	not done
adult female worms	13.70 ± 7.85 (2)	70.1
microfilariae	0.02 ± 0.002(2)	not done

[a] ng collagen solubilized at 25°C 24 h^{-1} (organism)$^{-1}$.
[b] Number of experiments.

TABLE III

Radioactivity released from native collagen by various tissues and enzymes

Material tested	No. of experiments	Percent radioactivity released	Percent inhibition by 10 mM EDTA
None	20	5.24 ± 2.35	–
A. aegypti			
salivary glands	1	12	0.0
hemolymph	1	10.24	0.0
Human skin (1 mm^3)	2	7.25	–
Peritoneal exudate fluid			
uninfected jirds	1	0	–
infected jirds	1	0	–
Trypsin (20 $\mu g\ ml^{-1}$)	2	12.24 ± 1.84	20.2
Collagenase			
R. catesbiana (40 $\mu g\ ml^{-1}$)	3	14.69 ± 2.55	98.9
C. histolyticum (20 $\mu g\ ml^{-1}$)	20	100	82.2

bers of *O. volvulus* microfilariae derived from skin snips, infective larvae of *B. malayi* isolated from infected *A. aegypti* mosquitoes and microfilariae or adult worms prepared from peritoneal cavities of *B. malayi*-infected jirds were added to tubes containing ^{3}H-labeled collagen. Vast differences in collagenolytic activity were observed among the various parasite stages tested. On a per worm basis, the highest activity was present in adult female *B. malayi*; *O. volvulus* microfilariae averaged eighty-fold higher enzyme activity than *B. malayi* microfilariae (Table II). The degree of inhibition by EDTA of enzyme activity associated with intact worms was much higher than that observed when worm extracts were used as the source of putative enzyme.

Components of host or vector origin that might contaminate these whole worm preparations also released radioactivity from collagen, but, in contrast to what was observed with mammalian or bacterial collagenase, this was not inhibited by EDTA (Table III). Thus, it is unlikely that host or vector derived contaminants account for the observed collagenolytic activity of the various parasite stages and extracts tested.

Finally, we determined whether enzyme activity was released by worms cultured in vitro. E-S products of all three stages of *B. malayi* tested contained detectable collagenolytic activity. Although adult female worms produce larger amounts of E-S proteins than infective larvae of *B. malayi* (average: 160 vs. 6.5 ng protein per 24 h per organism), the highest level of enzyme activity was present in E-S products of infective larvae (Table IV). During a 24 h in vitro incubation one larva produced sufficient enzyme to solubilize 8.7 ng collagen vs. 1.3 ng per adult worm.

TABLE IV

E-S products of *B. malayi* contain collagenolytic activity

Source of E-S products	Activity[a]	Percent inhibition by 10 mM EDTA
Infective larvae	1339 ± 535(5)[b]	60.4
Adult female worms	8.1 (2)	36.1
Microfilariae	5.3 (2)	43.5

[a] ng collagen solubilized at 25°C 24 h^{-1} (μg E-S protein)$^{-1}$.
[b] Number of experiments.

Characterization of collagenase present in filarial worms. The manner in which putative collagenase present in filarial worms acts on native collagen was examined by sequential analysis of the reaction products by SDS-PAGE. After 12 h of incubation, a considerable amount of intact soluble collagen (rather than fragments of low molecular weight) remained present. This indicates that solubilization of collagen molecules from preformed fibrils accounts for a portion of the radioactivity released from this substrate. After longer incubation periods (24–36 h), the overall

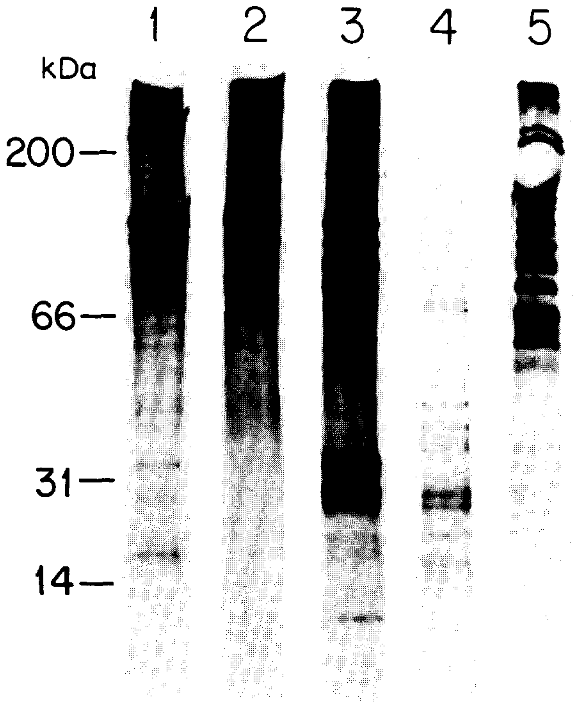

Fig. 1. Reaction products of filarial collagenase. ^{3}H-labeled-collagen was incubated with enzyme from various sources for 24–36 h. Supernatants from the incubation period were submitted to SDS-PAGE and the gels were autoradiographed as described in the text. Enzyme sources were E-S products of infective larvae of *B. malayi* (lane 1); buffer control (lane 2); *R. catesbiana* collagenase (lane 3); *C. histolyticum* collagenase (lane 4); *O. volvulus* adult worm extract (lane 5).

pattern of reaction products obtained with enzyme present in extracts of *O. volvulus* or *B. malayi* was similar to that produced by tadpole collagenase, (Fig. 1) but quite distinct from that obtained with a non-specific protease such as trypsin which produces many fragments of low molecular weight (results not shown).

The worm enzyme present in E-S products of infective larvae of *B. malayi* was inhibited by a number of substances that are known to inhibit other collagenases. The results of these studies are summarized in Table V.

Immunogenicity of filarial collagenase. The putative filarial collagenase is immunogenic in natural and experimental hosts of filariasis. Sera from patients with onchocerciasis or with brugian filariasis contain antibodies to E-S products of infective larvae of *B. malayi*, as well as to collagenase prepared from *C. histolyticum* (Table VI). In the case of onchocerciasis, serum titers of antibodies to *O. volvulus* antigens correlate well with titers of antibodies to *C. histolyticum* collagenase (r: 0.2987, $P < 0.05$). Immunization of mice with infective larvae of *B. malayi* also induces antibodies to *C. histolyticum* collagenase. More importantly, an IgM monoclonal antibody produced by the fusion of SP2-myeloma cells with spleen cells from a mouse hyperimmunized with adult *O. volvulus* extract not only reacts with soluble antigens of *O. volvulus* and of *B. malayi*, but also with purified *C. histolyticum* collagenase.

Additional studies provide evidence that the monoclonal antibody just described as well as antibodies present in sera of patients with oncho-

TABLE V

Effect of inhibitors on collagenolytic activity of E-S products of infective larvae

Inhibitor	Concentration	No. of experiments	Percent inhibition
STI	500 $\mu g\ ml^{-1}$	3	0
Fetal calf serum	16%	3	0
TLCK	1 mM	3	0
TPCK	1 mM	2	0
Platelet factor 4	50 $\mu g\ ml^{-1}$	3	0
PMSF	1 mM	4	64 ± 20
NEM	1 mM	4	38 ± 10
L-Cys	10 mM	3	58 ± 9
EGTA	10 mM	2	56 ± 16
EDTA (disodium)	10 mM	5	60 ± 15
Cu^{2+}	1 mM	3	93 ± 13
Zn^{2+}	1 mM	4	90 ± 14
pH	3.5	2	66

TABLE VI

Sera from patients with filarial infections contain antibodies to collagenase

Serum donor (origin)	Antibody titer to[a]	
	C. histolyticum collagenase	E-S products of *B. malayi* L3
Onchocerciasis (Venezuela)	0.37 ± 0.20 (71)	0.27 ± 0.18 (10)
Brugian filariasis (Indonesia)	0.58 ± 0.30 (34)	0.26 ± 0.24 (10)
Mansonellosis (Venezuela)	0.17 ± 0.16 (50)	0.09 ± 0.00 (9)

[a] Antibody titers were determined by ELISA as described in Methods. Titers are mean ± S.D. of Abs of test sera minus mean Abs of control sera. All sera were tested at 1:80 dilution. Values in parentheses are number of sera tested.

cerciasis and brugian filariasis react with collagenase. Incubation of *C. histolyticum* collagenase or E-S products of *B malayi* infective larvae with immunoglobulin-rich fractions of immune sera from patients or with the monoclonal antibody described above markedly reduces the enzyme activity of collagenase-containing preparations (Table VII). However, these antibodies do not appear to react with the active site of the enzyme, because the immune precipitate contains all enzymatic activity lost from the solution (results not shown).

To further investigate the similarity of bacterial and filarial collagenase, we compared the components identified by the monoclonal antibody to *O. volvulus* antigens no. 5 (OV5) on

TABLE VII

Immunogenicity of filarial collagenase

Antibody source	% decrease in collagenolytic activity	
	C. histolyticum	E-S of *B. malayi*
Normal human serum	28.0	12.2
Onchocerciasis serum	42.9	22.8
Brugian filariasis serum	52.7	89.8
SP-2 culture medium	23.2	12.9
Monoclonal antibody OV5	62.0	34.1
Affinity-purified antibodies from onchocerciasis serum	46.7	N.D.

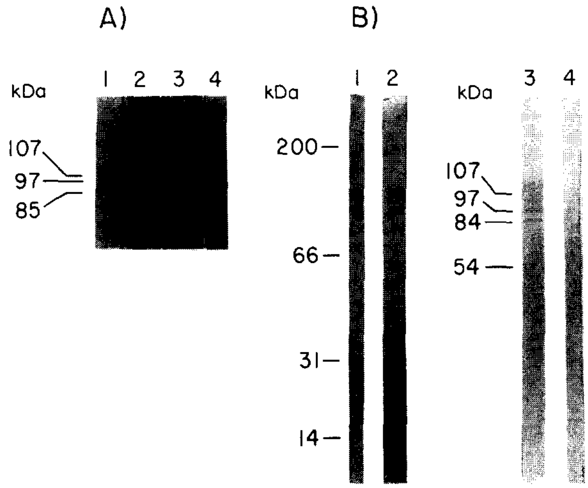

Fig. 2. Sera from donors with filarial infections contain antibodies to *C. histolyticum* collagenase. The figure shows Western blots of *C. histolyticum* collagenase separated on SDS-PAGE (5–20 % acrylamide) incubated with: (A) lane 1, serum from a normal nonimmune mouse; lane 2, serum from a mouse immunized with infective larvae of *B. malayi;* lane 3, serum from a patient with *O. volvulus;* lane 4, normal human serum; (B) lane 1, serum from a patient with onchocerciasis; lane 2, serum from patient with brugian filariasis; lane 3, monoclonal antibody OV5; lane 4, SP-2 supernatant.

Western blots of *C. histolyticum* collagenase, of E-S products of *O. volvulus* microfilariae and of adult female worms and infective larvae of *B. malayi*. The antibody reacted with four components of *C. histolyticum* collagenase preparations (est. 107, 97, 84 and 54 kDa), three components of *B. malayi* E-S (est. 107, 89 and 71 kDa) and four components of the *O. volvulus* E-S (est. 195, 135, 120 and 98 kDa). (Figs. 2, 3). Sera from patients with onchocerciasis or brugian filariasis likewise identified the 107, 97 and 84 kDa components of *C. histolyticum,* whereas sera from U.S.A. blood donors, from nonimmune mice and supernatants from SP2 myeloma cells do not react with *C. histolyticum* collagenase by this technique (Fig. 2).

Finally, to determine whether the putative filarial collagenase is a worm product or a contaminant of host origin, metabolically labeled E-S products of *B. malayi* infective larvae were immunoprecipitated with the monoclonal antibody and analyzed by SDS-PAGE. This antibody precipitated labeled E-S products of the similar mo-

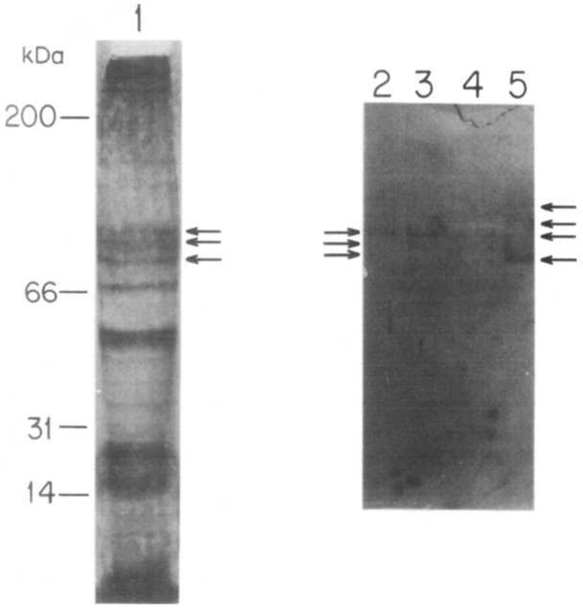

Fig. 3. Monoclonal antibody OV5 reacts with filarial antigens. Lane 1: autoradiograph of metabolically labeled E-S products of *B. malayi* infective larvae separated on SDS-PAGE (5–20 % acrylamide); Lanes 2–5: Western blot of E-S antigens incubated with monoclonal antibody OV5; lane 2: E-S products of adult *B. malayi;* lane 3: E-S products of infective larvae of *B. malayi;* lane 4: E-S products of *B. malayi* microfilariae; lane 5: E-S products of *O. volvulus* microfilariae.

lecular weights as those identified on Western blots (107, 89, 83, 71 kDa) (Fig. 4).

Discussion

The studies we report indicate that filarial nematodes contain an enzyme with collagenolytic activity. This enzyme solubilizes collagen molecules from native fibers and yields a pattern of reaction products similar to tadpole collagenase but distinct from a 'nonspecific' protease such as trypsin [23–27]. Thus, the filarial enzyme resembles a collagenase in its effect on this substrate. However, our assay system does not allow us to differentiate whether collagen, once solubilized, is further degraded by collagenase per se or by other proteases that may be present in the crude worm preparations. Experiments with enzyme inhibitors reveal that the putative filarial collagenase is readily inhibited by PMSF, NEM, L-Cys, EGTA and EDTA, by Cu^{2+} and Zn^{2+} ions and at low pH. Thus, enzyme activity was not inhibited by most inhibitors of serine esterases. In contrast, activity was inhibited by chelating agents and by some divalent cations (Cu^{2+} and Zn^{2+}). This suggests that the enzyme is a metalloprotease akin to vertebrate morphogenic collagenase but different from digestive collagenases of eukaryotes that often resemble trypsin (see ref. 27 for review). These results also indicate that filarial collagenase differs from acidic thiol proteases described in *Schistosoma mansoni* and *D. immitis* [9,28]. Inhibition by NEM and PMSF suggests the possibility that other proteases from filariae degrade collagen fragments partially hydrolysed by collagenase.

Several lines of evidence indicate that the collagenolytic activity we describe is a worm product, and not a contaminant derived from host or vector tissues. Extracts of salivary glands and hemolymph, constituents of mosquitoes that might

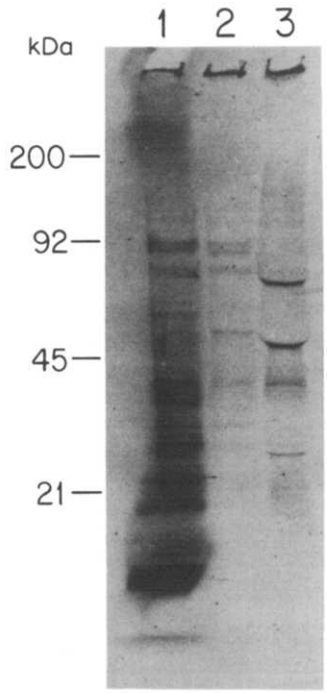

Fig. 4. Monoclonal antibody OV5 immunoprecipitates metabolically labeled E-S products from *B. malayi* infective larvae. An autoradiograph of metabolically labeled E-S products of *B. malayi* infective larvae separated on SDS-PAGE (5–20 % acrylamide) is shown in lane 1; lane 2 shows E-S products precipitated by monoclonal antibody OV5, lane 3 those precipitated by control human sera.

contaminate infective larvae, release only a small amount of radioactivity from labeled collagen, and this effect is not inhibitable by EDTA. Likewise, microfilaria-free skin from patients with onchocerciasis has negligible collagenolytic activity in our assay system. The same is true for peritoneal exudate fluid from infected and uninfected jirds. On the other hand, the unusually high collagenase activity observed in extracts of adult *O. volvulus* may be due in part to contamination with the bacterial collagenase used to digest the nodules. It should be pointed out, however, that bacterial enzyme was not used to generate any of the other filarial preparations that contain collagenase activity.

Studies with a monoclonal antibody provide direct evidence to suggest that the enzyme is a worm product. In Western blots, this antibody identifies an antigenic epitope present on a limited number of parasite molecules; it immunoprecipitates metabolically labeled E-S products of infective larvae of *B. malayi* and reduces collagenase activity in these preparations by precipitating the enzyme.

Of great interest is the observation that worm collagenase is immunogenic in natural and experimental hosts of filariasis. Sera from patients with onchocerciasis and brugian filariasis contain antibodies to this enzyme. These also develop in mice immunized with live infective larvae of *B. malayi*. The human antibodies and monoclonal antibody OV5 produce similar patterns of reaction in Western blots of *C. histolyticum* collagenase and immunoprecipitate moieties of similar molecular weight from this preparation. This suggests that the antigenic sites recognized by human antibodies are on the same polypeptides as the epitope identified with the monoclonal antibody and indicates that the bacterial and filarial enzyme share antigenic determinants. On the other hand, Western blot analysis of collagenase from *B. malayi* and from *O. volvulus* with monoclonal antibody OV5 reveals differences in the apparent molecular weight of collagenase prepared from these two filarial species. For lack of sufficient *O. volvulus* material, it was not possible to determine whether this is associated with differences in other properties of the enzymes.

At present, we can only speculate about the biological role, if any, of filarial collagenases. A complex set of molecules is released by filarial worms maintained in vitro, even when the worms remain viable and motile, as was the case in our experiments [29]. Whether such E-S products merely leak out of dying worms or are actively secreted in vitro and in vivo remains unclear, despite the traditional name for these parasite materials. Nevertheless it is of interest that microfilariae of *O. volvulus* contain higher enzyme activity than those of *B. malayi* and that E-S products of infective larvae contain higher levels of collagenase than E-S products of other stages. These observations suggest a possible role of this enzyme in the migration of filarial worms through tissues. Adult *O. volvulus* reside mainly within onchocercomata, subcutaneous nodules with a fibrous outer surface. It is possible that microfilariae escape from these nodules and migrate through the skin by local proteolysis of extracellular matrix, mediated in part by worm collagenase. Conceivably, the pronounced atrophy of the skin observed in patients with long-standing microfilarodermia could be the end-result of the same pathogenic process. Decreased cross-linking of collagen in tissues infected with *B. malayi* has been reported [30]. Skin penetration of infective larvae likewise would be aided by collagenolytic enzymes. A similar role has been ascribed to proteinases of *Strongyloides* species and of *S. mansoni* [5–7].

A second possible biological function of filarial collagenase could be to maintain the integrity of the worms' cuticle, which contains collagen in most nematodes. This might be especially important during the moulting process, when the existing cuticle is replaced by a new one. Lastly, digestion of host collagen may provide these worms with a ready source of amino acids needed for the parasites' own requirements. Experiments to evaluate these possibilities are in progress.

Acknowledgments

This work was supported by a fellowship from the Fundacion Gran Mariscal de Ayacucho to Izaskun Petralanda and grant AI 16479 from the National Institutes of Health. The authors are grateful to Bruce Hamill and the personnel of

CAICET for their technical support, to the Yanomami of Sierra Parima for their enthusiastic participation in the field stages of this project, to Ramona Gonski for her skillful typing of this manuscript, to Dr. Paula Bockenstedt for her generous gift of purified platelet factor 4, and to Drs. J. Ribeiro and T. Mather for the gift of *A. aegypti* haemolymph and salivary glands.

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